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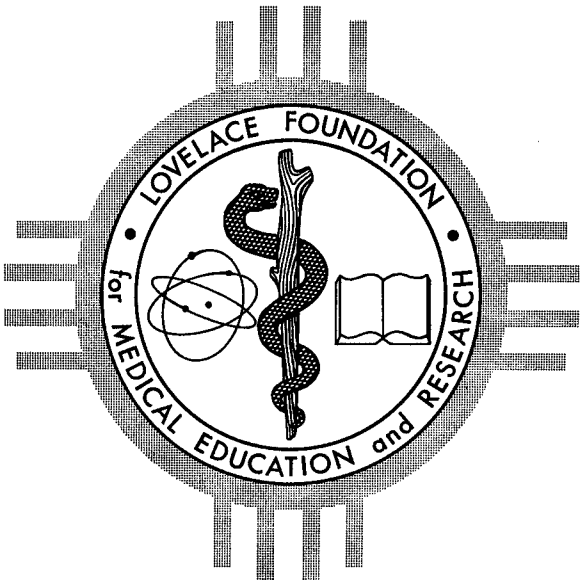

THE FLORA OF HEALTHY DOGS

I. BACTERIA AND FUNGI OF THE NOSE, THROAT, AND LOWER INTESTINE

by

W. E. CLAPPER AND G. H. MEADE

Albuquerque, New Mexico

September, 1962

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by
W. E. Clapper and G. H. Meade

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From the Department of Microbiology
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ABSTRACT

Practical methods for isolating and identifying organisms in the lower intestine, nose, and throat of the dog were outlined and employed. Using these, bacteria and fungi from rectal swabs were determined at two different times on 22 dogs. Results indicated that the flora was relatively stable with wide fluctuations in only a few groups and these, largely, were in those bacteria less frequently observed. E. coli and S. viridans were the organisms most frequently found.

S. viridans and Neisseriae were most often isolated from throat swabs and coagulase negative staphylococci, S. viridans, and Neisseriae from nasal swabs. The flora from all areas was similar to that found in humans. All organisms cultured were identified, giving a total of 49 different bacteria and fungi.

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SUMMARY

Rectal swabs taken at two different periods on 22 Beagles were each inoculated to 11 primary isolation media. Twenty species of bacteria and ten species of fungi were isolated and identified. The organisms were similar to those found in the human intestine. E. coli, S. viridans, enterococci, S. lactis, Bacillus species, and coliforms other than E. coli were most frequently encountered. The frequency of occurrence was approximately the same at both samplings in the more commonly cultured bacteria, with the exception of S. lactis and Bacillus species. Pathogenic E. coli were isolated from nearly one-third of the first specimens. These were the only human pathogens observed.

Throat and nasal swabs were taken from 25 dogs and each was inoculated to ten primary isolation media. Twenty-nine species of bacteria and two species of yeasts were identified in the throat cultures and 27 species of bacteria were identified from the nasal cultures. Streptococcus viridans, Neisseria, and coagulase negative staphylococcus were most often isolated. The flora was similar to that found in human nose and throat cultures, except that more Hemophilus and pneumococcus, and fewer coliforms, are generally found in human throats. Organisms resembling human pathogens were group A streptococci and coagulase positive staphylococci. These were isolated infrequently.

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I. INTRODUCTION

It is well known that animals exposed to large doses of x-irradiation often succumb to an overwhelming septicemia due to organisms commonly found in the intestinal tract (1), and organs normally sterile in healthy animals are found to be infected with these bacteria when examined at autopsy (2). Generalized infection after irradiation has been observed in man (1). An increase in coliform bacilli in the intestines of x-irradiated dogs was reported by Furth et al. (3), and an increase in coliforms, accompanied by a decrease in lactobacilli in the intestines of irradiated rats, was observed by Bell et al. (4).

As an aid in establishing the cause of disease and death in dogs exposed to ionizing radiation, activities were initiated to identify the bacterial and fungal flora of those areas most likely to contribute causative organisms. Though studies of bacteria found in certain areas of the dog's intestinal tract (5-10) have been made and large numbers of dogs have been investigated to determine the incidence of specific organisms (11), reports of attempts to isolate all the bacteria and fungi present in the lower intestine, nose, and throat of dogs are either rare or have not been published.

The purpose of this paper is twofold; namely, to report the organisms found in 25 healthy dogs and to outline the methods employed, since they may be of practical use to those interested in radiation effects studies or in following the changes in the bacterial and fungal flora of animals subjected to a variety of other experimental variables.

II. METHODS

A. Specimens

1. Rectal Swabs

In collaboration with personnel of the Section of Veterinary Medicine, rectal swabs on 22 Beagles were taken during the period from September 7, 1961 to October 9, 1961. Three of these dogs were not available for the second study during the period from October 9, 1961 to November 20, 1961, but an additional three were added to the group, making a total of 22. The swabs were plastic enclosed rectal swabs (Falcon) designed to prevent contamination from the outer areas of the anus. These were

immediately placed in two ml of Proteose Peptone No. 3 broth (Difco). Gram stains were made from a second specimen on the first eight dogs studied. The media listed in Table 1 was inoculated from the broth. Chocolate and blood agar plates were incubated in 10 per cent CO_2. The general scheme employed for primary isolation is also presented in Table 1. All colonies on each plate which appeared morphologically different were subcultured to appropriate media in order to have pure cultures. They were identified by Gram stains and by biochemical and serological studies.

2. Nose and Throat Swabs

The tonsillar areas and the anterior nares of 25 dogs were swabbed and the swabs placed in broth. Primary isolation was made in the media listed in Table 1. Individual colonies were picked from each medium for isolation in pure culture and were then identified by further studies as set forth in the following section. Selective media are indicated in Table 1.

B. Criteria for Identification of Organisms

The criteria employed for identifying the organisms isolated are presented below in outline form.

1. Enteric bacilli – gram negative

a. Lactose fermenters in 24 hours. Red colonies on desoxycholate, MacConkey, or Salmonella-Shigella (SS) agar.

Organism	H_2S*	Indole	M.R.**	V.P.	Ci.
E. coli	-	+	+	-	-
E. freundii	+	v	+	-	+
A. aerogenes	-	-	-	+	+
Intermediate coliforms	vary from the first three				

*H_2S was determined with the triple sugar iron medium (TSI).

**M.R., V.P., and Ci. refer to the methyl red, Voges-Proskauer, and citrate utilization tests.

v indicates variable reaction.

Adapted from Schaub, I. G., Foley, M. K., Scott, E. G., and Bailey, W. R.: Diagnostic Bacteriology, 5th edition, C. V. Mosby Co., St. Louis, 1958 (12).

TABLE 1

MEDIA USED FOR THE PRIMARY ISOLATION OF MICROORGANISMS FROM THE NOSE, THROAT, AND INTESTINE OF THE DOG

Medium	Use	R*	N & T**
Aerobic:			
T-soy blood agar***	Total flora	X	X
Phenylethyl alcohol agar	Gram positive cocci	X	X
Tomato-juice agar	Lactobacilli, staphylococci yeasts	X	X
Desoxycholate agar	Enterics	X	X
Salmonella-Shigella agar	Enteric pathogens	X	
Selenite broth	Enteric pathogens	X	
Chocolate agar	Fastidious organisms, Neisseria, Hemophilus		X
Staph. medium No. 110 agar	Staphylococci		X
Cystine trypticase agar	Fastidious organisms, Neisseria, Hemophilus		X
Anaerobic:			
T-soy blood agar	Anaerobes	X	X
Thioglycollate medium	Anaerobes	X	X
Fungus:			
Sabaraud's dextrose agar	Aerobic fungi	X	
Mycosel agar	Aerobic fungi, pathogens	X	
Anaerobic blood agar slant	Actinomyces	X	X

*Rectal swab
**Nose and throat swab
***Outdated blood bank blood

Swabs placed in Proteose Peptone No. 3 broth and media inoculated from this.

b. Lactose fermenters in 48 hours or more

Organism	H_2S	Indole	M. R.	V. P.	Ci.
Paracolobactrum coliformis	-	+	+	-	-
Paracolobactrum aerogenoides	-	-	-	+	+
Paracolobactrum intermedium	vary from the first two				

c. Non-lactose fermenters. Colorless colonies of Gram negative bacilli found on desoxycholate, MacConkey, or SS agar. Inoculated to triple sugar iron medium (TSI)* slants and identified by the following scheme. Selenite broth was streaked to a MacConkey and an SS agar plate and colorless colonies on these plates were treated in the same manner.

1) TSI Group +/+ (acid slant, acid butt)

a) Urea positive

Proteus (species identification as indicated below)

Biochemical Identification of Proteus

Organism	H_2S on TSI	Indole	Citrate
Proteus mirabilis	+	-	+
Proteus vulgaris	+	+	-
Proteus morganii	-	+	-
Proteus rettgeri	-	+	+

b) Urea partial, or urea negative

Paracolon

2) TSI Group -/- (alkaline slant, alkaline butt)

a) Honey-like odor and/or greenish pigment on desoxycholate or brain heart infusion slants

Pseudomonas aeruginosa

*Baltimore Biological Laboratories

b) None of the above characteristics

(1) Reduce nitrates and alkalinize litmus milk

Motile: Alcaligenes fecalis

Non-motile: Alcaligenes metalcaligenes

(2) Do not reduce nitrates

(a) Ferment 10 per cent lactose agar slant

Mima polymorpha (Bacterium anitratum – oxidase positive and negative varieties)

(b) Do not ferment 10 per cent lactose

Achromobacter (colorless colonies)

Flavobacterium (yellow colonies)

The Achromobacter group would include: Bordetella parapertussis and Bordetella bronchiseptica in Diagnostic Bacteriology's scheme, but, for convenience, these were not further identified.)

3) TSI Group -/+ (alkaline slant, acid butt)

a) Urea positive

Proteus

b) Urea positive, partially

Paracolon

c) Urea negative

Shigella, biochemically according to reactions shown in Table 2. Confirmed by slide agglutination with group-specific antiserum

Salmonella, biochemically as shown in Table 2; confirmed by polyvalent Salmonella antiserum

Paracolon, those which were not Shigella or Salmonella either biochemically or serologically.

d. Pathogenic E. Coli

Three colonies of E. coli from each specimen were inoculated to brain heart infusion slants. After incubation for 24 hrs, they were tested by slide agglutination with

TABLE 2

TYPICAL REACTIONS OF VARIOUS ORGANISMS ON DIFFERENTIAL TUBE MEDIA*

Organism	Purple broth base containing						Indole	Motility	H_2S	Citrate
	Dextrose	Maltose	Sucrose	Lactose	Mannitol	Salicin				
Shigella dysenteriae	Y	NC	NC	NC	NC	NC	-	-	-	-
Shigella ambigua	Y	NC	NC	NC	NC	NC	+	-	-	-
Shigella sonnei	Y	Y	Y	Y slow	Y	NC	-	-	-	-
Shigella paradysenteriae	Y or YG	V	NC	NC	Y or YG	NC	v	-	-	-
Shigella alkalescens	Y	Y	NC or Y	NC	Y	NC	+	-	-	-
Shigella madampensis	Y	Y	Y	Y	Y	NC	+	-	-	-
Shigella dispar	Y	Y	Y	Y	Y	NC	+	-	-	-
Salmonella typhosa	Y	Y	NC	NC	Y	NC	-	+	+	-
Salmonella paratyphi	YG	YG	NC	NC	YG	NC	-	+	-	-
Salmonella schottmuelleri	YG	YG	NC	NC	YG	NC	-	+	+	+
Salmonella typhimurium	YG	YG	NC	NC	YG	NC	-	+	+	+
Salmonella choleraesuis	YG	YG	NC	NC	YG	NC	-	+	-	+
Salmonella enteritidis	YG	YG	NC	NC	YG	NC	-	+	+	+
Salmonella pullorum	YG	NC	NC	NC	YG	NC	-	-	+	-
Salmonella gallinarum	Y	Y	NC	NC	Y	NC	-	-	v	-

NC = no change or alkaline reaction
Y = yellow-acid formation
YG = acid and gas formation

\+ = positive for a given reaction
\- = negative

Rapid identifying characteristics: acid and gas from dextrose and indole positive or acid from salicin

Paracolon

*From Difco Manual, 9th edition, pg. 160.

polyvalent OB antiserum groups A and B (antiserum produced by Difco Laboratories). If positive, they were identified with specific typing antiserum.

2. Other Gram negative bacilli and Gram negative diplococci (isolated from aerobic trypticase soy blood agar, chocolate agar, or cystine trypticase agar)

   a. Gram negative diplococci (species identification by reaction, in table below)

      Neisseria species

      Mima polymorpha

      Herella vaginicola

      Colloides anoxydana

Species Differentiation of Neisseriae and Mimae

Organism	Growth on agar	Rods and filaments in broth	Oxidase	Fermentation of Dextrose	Maltose	Sucrose
Neisseria gonorrheae	-	-	+	+	-	-
Neisseria meningitidis	-	-	+	+	+	-
Neisseria catarrhalis	+	-	+	-	-	-
Neisseria pharyngis gp.	+	-	+	+	+	v
Mima polymorpha	+	+	-	-	-	-
Mima polymorpha var. oxidans	+	+	+	-	-	-
Herella vaginicola	+	+	-	+	-	-
Colloides anoxydana	+	+	-	+	+	-

Compiled from Schaub, I. G., Foley, M. K., Scott, E. G., and Bailey, W. R.: Diagnostic Bacteriology, 5th edition, C. V. Mosby Co., St. Louis, 1958 (12); Gangarosa, E. J. and Cary, S. G.: Validity of reports of penicillin-resistant gonococci. J. A. M. A., 173: 1808-1810, 1960 (13); Brooks, B. E. and Sanders, A. C.: Unidentified Gram negative rods and the tribe Mimae. U. S. A. F. Med. J., 5: 667-672, 1954 (14).

   b. Gram negative bacilli (microscopically small rods)

      1) No growth on desoxycholate agar; satellite formation on blood agar with Staphylococcus aureus

         Hemophilus

            Hemophilus influenzae (satellitism on nutrient agar without blood with S. aureus, but not with S. fecalis)

Hemophilus parainfluenzae (satellitism with S. fecalis)

Hemophilus hemolyticus (hemolytic on blood agar)

2) Small Gram negative bacilli which were not Hemophilus were tested biochemically and identified as Pasteurella multocida (septica) or Pasteurella pseudotuberculosis according to the characteristics outlined for these organisms in Topley and Wilson (15). No further identification was attempted.

3. Gram positive bacilli

a. All large Gram positive bacilli which formed a pellicle in broth

Bacillus subtilis

b. Small rods; catalase positive; species identified by biochemical characteristics outlined in Bergey's Manual (16)

Corynebacterium

c. Small, slow-growing colonies on blood agar; catalase negative; grow well on tomato juice agar

Lactobacillus

4. Gram positive cocci

a. White or orange shiny colonies; catalase positive

1) Opacity around colonies streaked on plasma agar—coagulase positive staphylococci

2) No opacity around colonies—coagulase negative staphylococci

b. Small opaque to clear colonies; catalase negative

Streptococcus

Identifying Characteristics of the Streptococci

Organism	B	a	MB	NaCl	P	A
Enterococcus	v	v	+	+	-	-
Streptococcus lactis	-	v	+	-	-	-
Streptococcus viridans	-	+	-	-	-	
Pneumococcus	-	+			+	
Beta streptococcus gp. A	+	-	-	-		+
Other beta streptococcus	+	-	-	-		-

v = variable; B = clear hemolysis on blood agar; a = green hemolysis on blood agar; MB = growth in 0.1 per cent methylene blue milk; NaCl = growth in 6.5 per cent NaCl broth; P = zone of inhibition with optochin* disk; A = zone of inhibition with bacitracin* disk.

5. Anaerobes

   a. All Gram negative bacilli which would not grow aerobically

      Bacteroides

   b. Gram positive cocci which would not grow aerobically

      Anaerobic streptococci

   c. Large Gram positive bacilli which would not grow, or grew very poorly, aerobically; species identification was made by the characteristics listed in Bacterial and Mycotic Infections in Man (17)

      Clostridium

   d. Rough, piled up colonies on blood agar which would not grow aerobically; thin, pleomorphic Gram positive rods

      Actinomyces

6. Fungi

   a. Microscopically large, round or oval organisms; inoculated to chlaymdospore media (Difco) and corn meal agar

      Yeasts

      1) Candida albicans (produce chlamydospores)

*Taxos P and A disks produced by Baltimore Biological Laboratories.

2) Other yeasts (do not produce chlamydospores)

b. Identified by cultural and microscopic characteristics as outlined in Manual of Clinical Mycology, 2nd edition (18)

Molds

III. RESULTS

The individual species isolated from each area are listed in Table 3. The data will be discussed according to the origin of the organisms.

A. Rectal Swabs

Gram stains often revealed a non-culturable spirillum or spirochete, a finding which has been observed by others (6, 19).

The percentage of dogs in which the various kinds of bacteria were found is shown in Figure 1 for the two series of experiments performed. E. coli and Gram positive cocci – Streptococcus viridans, enterococci, and Streptococcus lactis – were most prevalent. With the exception of Streptococcus lactis, there was little difference in the frequency of these organisms in the two samples taken from the same dogs at different times. This is true also for the other enteric bacilli: Paracolon, Aerobacter, Proteus, and Pseudomonas. Pathogenic E. coli, however, were observed in many more of the dogs during the first period of testing than at the later period (see Figure 1). There was a large difference in Bacillus species which probably has little significance in relation to the health of the animal and is most likely related to the flora of the environment. Proteus were present in approximately one-third of the specimens at both times. Proteus mirabilis was the species most often isolated. Coagulase negative staphylococci and Pseudomonas were seen in only a small per cent and in the same number at both test periods. No coagulase positive staphylococci were isolated. Clostridia and Lactobacilli were of low prevalence and both were found less often in the first specimens than in the second. A variety of non-pathogenic fungii were isolated; Mucor species were most prevalent, being noted in eight specimens.

B. Throat Swabs

The percentage of dogs in which the various kinds of bacteria were isolated from throat swabs is shown in Figure 2. Streptococcus viridans and Neisseriae predominated in all the animals. The next most frequently

TABLE 3

MICROORGANISMS ISOLATED FROM TWENTY-FIVE BEAGLES

(Listed in Order of Frequency)

Nose	Throat	Rectum
Coag. neg. staphylococcus	Streptococcus viridans	Escherichia coli
Streptococcus viridans	Coag. neg. staphylococcus	Streptococcus viridans
Streptococcus lactis	Neisseria flavescens	Enterococci
Neisseria flavescens	Neisseria pharyngis	Streptococcus lactis
Bacillus species	Escherichia coli	Aerobacter aerogenes
Neisseria catarrhalis	Streptococcus lactis	Bacillus species
Mima polymorpha	Bacillus species	Paracolon
Enterococcus	Alcaligenes fecalis	Intermediate coliform
Pseudomonas aeruginosa	Mima polymorpha	Proteus mirabilis
Corynebacterium enzymicum	Neisseria catarrhalis	Pathogenic Escherichia coli
Aerobacter aerogenes	Pseudomonas aeruginosa	Escherichia freundii
Neisseria pharyngis	Aerobacter aerogenes	Clostridium perfringens
Lactobacillus	Lactobacillus	Bacillus subtilis
Clostridium perfringens	β streptococcus (not gp A)	Coag. neg. staphylococcus
Escherichia coli	Intermediate coliform	Pseudomonas aeruginosa
Paracolobactrum intermedium	Paracolobactrum intermedium	Lactobacilli
Corynebacterium bovis	Group A streptococcus	Proteus vulgaris
Bacillus subtilis	Corynebacterium bovis	Proteus morganii
Alcaligenes metalcaligenes	Enterococcus	Pseudomonas species
Corynebacterium hoagii	Clostridium perfringens	Coag. pos. staphylococcus
Coag. pos. staphylococcus	Coag. pos. staphylococcus	Mucor
Alcaligenes fecalis	Pasteurella septica	Fusarium
Corynebacterium hofmanni	Corynebacterium diphtheriae	Hormodendrum
Intermediate coliform	(non-toxigenic)	Diplosporium
Clostridium septicum	Corynebacterium enzymicum	Geotrichum
Hemophilus hemolyticus	Corynebacterium striatum	Penicillium
Paracolon	Achromobacter	Cyncephalastrum
	Paracolon	Oospora
	Proteus mirabilis	Candida albicans
	Yeast	Yeast
	Candida albicans	

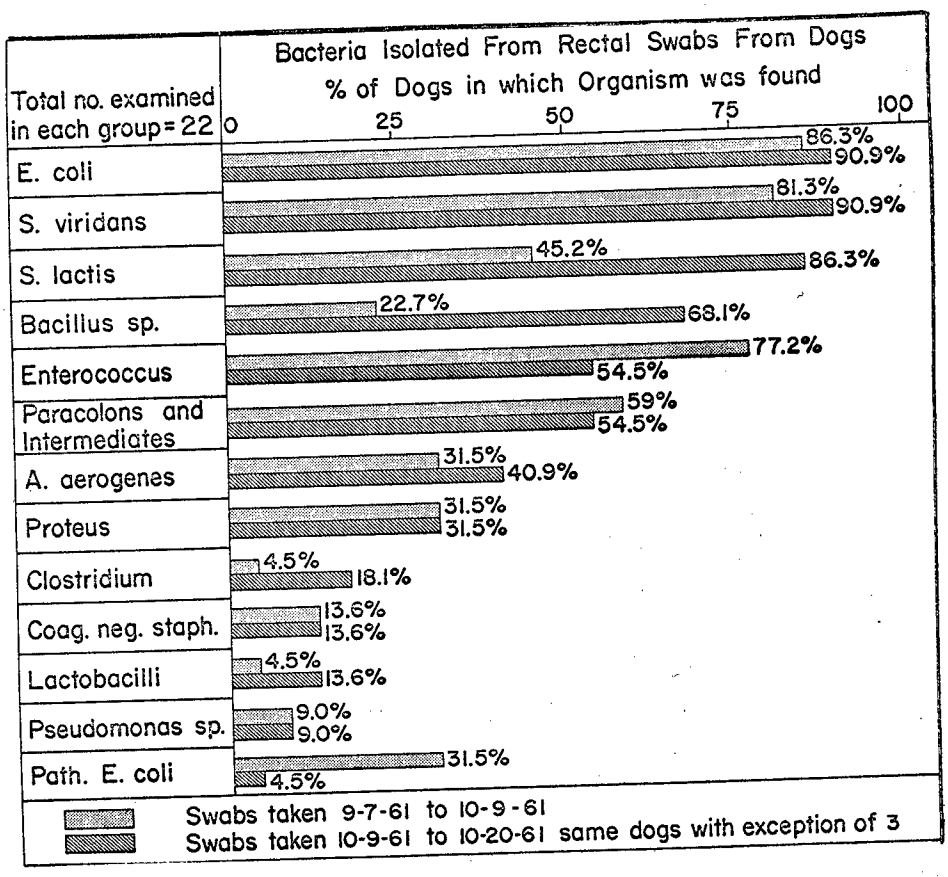
Total no. examined in each group = 22
Bacteria Isolated From Rectal Swabs From Dogs
% of Dogs in which Organism was found
0
25
50
75
100
E. coli
86.3%
90.9%
S. viridans
81.3%
90.9%
S. lactis
45.2%
86.3%
Bacillus sp.
22.7%
68.1%
Enterococcus
77.2%
54.5%
Paracolons and Intermediates
59%
54.5%
A. aerogenes
31.5%
40.9%
Proteus
31.5%
31.5%
Clostridium
4.5%
18.1%
Coag. neg. staph.
13.6%
13.6%
Lactobacilli
4.5%
13.6%
Pseudomonas sp.
9.0%
9.0%
Path. E. coli
31.5%
4.5%
Swabs taken 9-7-61 to 10-9-61
Swabs taken 10-9-61 to 10-20-61 same dogs with exception of 3

Figure 1

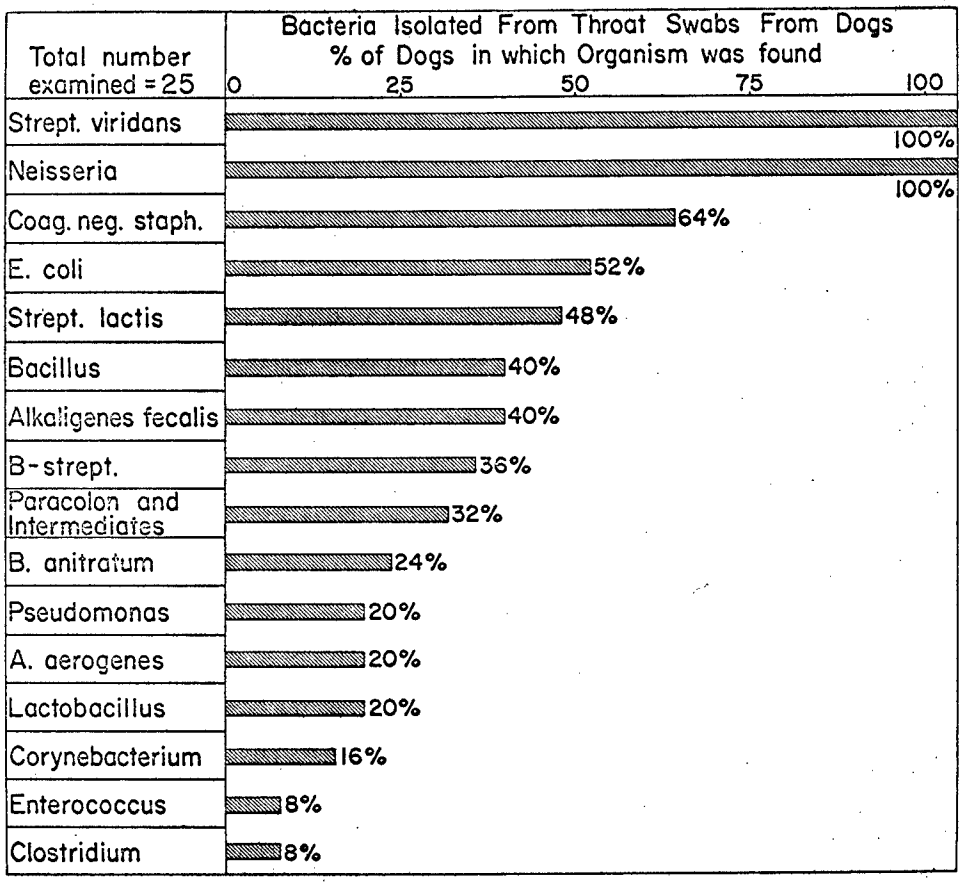
Total number examined = 25
Bacteria Isolated From Throat Swabs From Dogs
% of Dogs in which Organism was found
0
25
50
75
100
Strept. viridans
100%
Neisseria
100%
Coag. neg. staph.
64%
E. coli
52%
Strept. lactis
48%
Bacillus
40%
Alkaligenes fecalis
40%
B-strept.
36%
Paracolon and Intermediates
32%
B. anitratum
24%
Pseudomonas
20%
A. aerogenes
20%
Lactobacillus
20%
Corynebacterium
16%
Enterococcus
8%
Clostridium
8%

Figure 2

observed bacteria were the coagulase negative staphylococci. Coagulase positive staphylococci were isolated from only two animals and so were not included in the graph. About 50 per cent of the dogs were noted to have E. coli in the throat with other enteric bacilli seen less frequently. Thirty-six per cent carried beta hemolytic streptococci, nearly one-half of which were group A. Lactobacilli, Corynebacterium species, and enterococci were present, but in small numbers.

This flora would seem to be very similar to that which one of the authors (W. E. C.) has observed over a period of ten years in a clinic diagnostic laboratory where several throat swabs from humans of all ages are cultured daily. The greatest difference was in the lack of Hemophilus species and pneumococci in dogs and which are commonly encountered in humans, and the greater number of enterics found in the dogs. Proteus species are not often cultured from throats of humans; they were infrequently isolated from the throats of the dogs.

C. Nasal Swabs

Figure 3 shows the results of the study carried out on nasal swabs. The organisms most frequently isolated were the same as those noted in the throat, except that the coagulase negative staphylococci were found in every dog; coagulase positive staphylococci were not encountered. Corynebacterium species were seen frequently. The coliform bacilli were much less in evidence in the nasal swabs than in the throat swabs. Beta hemolytic streptococci and pneumococci were not observed. Bacillus subtilis or related organisms were apparent in about one-half of the cultures, and this was true of both throat and rectal swabs.

IV. DISCUSSION

Bacterial counts were not attempted because it was felt that this would complicate a study involving a large number of animals for an extended period of time to the point of diminishing productivity in results. Furthermore, Smith (6) abandoned such counts because they varied greatly in the same dog and had no apparent relation to the health of the animal. Since Bornside et al. (10) stated that the most commonly found bacteria were also the most numerous in both control groups and dogs with closed loops in the intestine, it seemed justifiable in the present study to use the percentage occurrence as a measure of the prevalence of different species or groups.

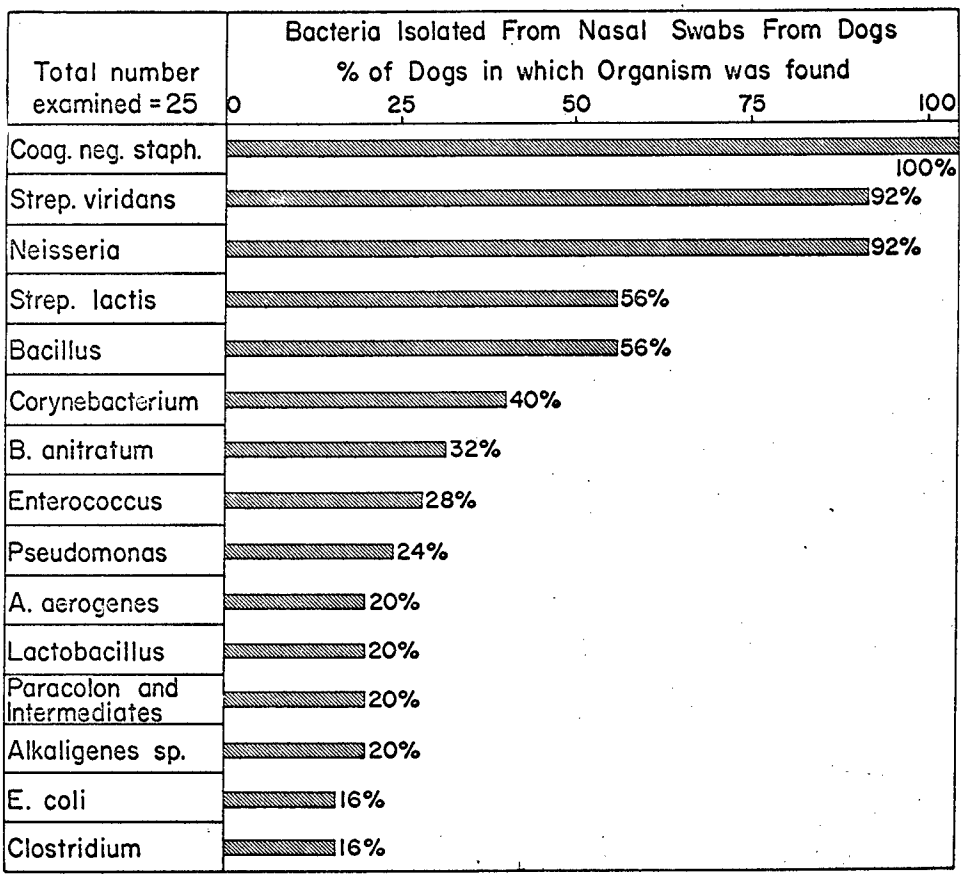
Total number examined = 25
Bacteria Isolated From Nasal Swabs From Dogs
% of Dogs in which Organism was found
0
25
50
75
100
Coag. neg. staph.
100%
Strep. viridans
92%
Neisseria
92%
Strep. lactis
56%
Bacillus
56%
Corynebacterium
40%
B. anitratum
32%
Enterococcus
28%
Pseudomonas
24%
A. aerogenes
20%
Lactobacillus
20%
Paracolon and Intermediates
20%
Alkaligenes sp.
20%
E. coli
16%
Clostridium
16%

Figure 3

Specimens showing any species in nearly pure culture would be given the same significance as possible causes of disease as they are in a clinical microbiology laboratory. Zubrzycki and Spaulding (20) have recently reported a study in which they found the normal human fecal flora to be remarkably stable. Wide fluctuations occurred only in the number and type of less frequently observed organisms. They believed that a significant variation in the normal flora would affect the health of the individual. A comparison of the results of two specimens taken at different times on the same dogs (Fig. 1) showed little variation in most of the more commonly isolated organisms with the exception of Streptococcus lactis. It seems probable that the fecal flora of healthy dogs is also quite stable.

Smith (6), in her study of the bacterial flora of isolated segments of the small intestine, examined 307 specimens from 40 dogs. No attempt was made to isolate and identify all organisms as was done in this study, but a comparison of results is of interest. Clostridium welchii was found in 87 per cent of the specimens and B. coli in 85 per cent. Non-hemolytic streptococci (45 per cent) and hemolytic streptococci were next in order. In the present study, Clostridium were noted much less often, perhaps because the sampling was made from the lower intestine.

Haenel and Mueller-Beuthow (21) examined the fecal flora of several animals including dog and man. Two specimens were cultured from each subject, six times in four weeks. The flora of man and dog were observed to be quite similar and to consist of aerobes, anaerobes, coliforms, and enterococci in that quantitative order. Staphylococci were rare. Data reported in the present study confirm the staphylococcal findings and coliforms, enterococci, and lactobacilli proved to be among the six most frequently cultured organisms.

Mikhlin and Geimberg (22) reported the fecal flora of dogs to consist chiefly of acidogenic streptococci, coliforms, and lactic bacilli, and another group (9) stated that E. coli was the most common organism. In the survey reported in this paper, E. coli, S. viridans, S. lactis, and enterococci were the bacteria isolated most often. Since Zubrzycki and Spaulding (20) found Bacteroides to be the predominating organism in human feces, in spite of coliforms being so considered by others, it might be useful in future studies to make dilutions as they did in order to allow better isolation of these

smaller and slower-growing colonies. It would seem doubtful, however, that this would be of importance in evaluating the health of an animal unless they suddenly appeared as the predominating organism of the culture with the usual flora suppressed or absent.

Proteus species, identified in one-third of the specimens examined in the present study, have been isolated by others (6, 10, 11, 23) and Proteus is generally considered to be part of the normal flora. Gebert (24), however, found none in healthy dogs, but did note P. mirabilis and P. morganii in 60 per cent of those with dysentery.

Although several extensive investigations have shown dogs to be carriers of Salmonella (9, 11, 25), neither Salmonella nor Shigella were found in the current study. The most interesting finding related to the dog as a carrier of human pathogens, as noted in Figure 1, was the isolation of several pathogenic E. coli. Mian (26) has reported that dogs carry pathogenic E. coli in their intestines and may be a source of infection to man. Seven of the 22 dogs first examined here (see Fig. 3) were carrying these organisms. Five were type 0119B14, and two were type 055B5. Only one dog was still carrying a pathogenic E. coli when examined the second time. This was type 055B5.

Frequent isolation of Pasteurella multocida from the tonsils and nose of healthy dogs has been reported (27). It has been occasionally noted in dog bites (28, 29). Beta hemolytic streptococci have also been cultured from dog tonsils, none of which were human type strains (30, 31). Mann (32) reported that 23 of the nasal swabs from 100 dogs yielded coagulase positive staphylococci. Pasteurella multocida was found in only two of the throat and one of the nasal swabs in the 25 dogs examined in the present study. There were nine throat swabs that showed beta hemolytic streptococci, four of which were group A by the bacitracin disk test (33). These were not identified serologically. No beta hemolytic streptococci and only two coagulase positive staphylococci were isolated from the nasal swabs.

It is evident that certain differences will be found in the intestinal and respiratory flora of dogs, depending upon the methods used for isolation, the manner in which cultures are taken, details relevant to housing, and, perhaps, the contribution of other factors. However, in general, the same organisms have been noted in the various studies reviewed above, and the flora does not

seem to differ greatly from that of humans. Therefore, it appears feasible to use the methods outlined in this paper to determine major fluctuations in a variety of experimental situations including exposure to radiation. To correlate this with the dog's health under various experimental conditions, it will be necessary to conduct similar determinations on a control as well as on the experimental group. Although the methods outlined are not new, it is hoped that the organization of them will be useful to others interested in making similar evaluations of bacterial flora.

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